

CLAIMS

1. Process for obtaining a fragment of bovine DNA which has a defined size and sequence and is specific to bovines, and in particular the species *Bos taurus* and *Bos indicus*, from a sample of organic matter, and a process by which a defined sequence of the bovine genome present in bovine genomes but absent from the genomes of other animal species is amplified by a polymerase chain reaction.

10 2. Process for detection and identification of the presence of biological matters of bovine origin in a sample of organic matter, characterized in that the presence of DNA of bovine origin is determined in the said organic matter by amplification of a specific DNA sequence of the bovine genome.

15 3. Process according to ~~claims 1 and 2~~, characterized in that the amplified sequence of the bovine genome is of mitochondrial origin.

4. Oligonucleotides, characterized

20 - in that they have a sequence identical to the extent of at least 80%, preferably 90%, and advantageously 95%, to an oligonucleotide made up of a sequence of about 15 to 25 nucleotides, in particular 20 to 25 nucleotides, contained in the following SEQ ID No. 1:

TAATGTCCATGCTTATCATTATGCTGGTGCTCAAGATGCAGTT

- or in that they comprise the following sequence ID No. 2:

25 YTATCATTATGCTGG

- or in that they are made up of the following sequence ID No. 3:

CATGCYTATCATTATGCTGG

in which Y is T or C.

30 5. Oligonucleotides, characterized

- in that they have a sequence identical to the extent of at least 80%, preferably 90%,

and advantageously 95%, to an oligonucleotide made up of a sequence of about 15 to 25 nucleotides, in particular 20 to 25 nucleotides, contained in the following SEQ ID No. 4:

ATTATATGCCCATGCATATAAGCAAGTACATGACCTCTATAGCAG

5 - or in that they comprise the following sequence ID No. 5:

TAAGCAAGTACATGA

- or in that they are made up of the following sequence ID No. 6:

GCATATAAGCAAGTACATGA

10 6. Pairs of primers, characterized in that they are made up of one or other of the oligonucleotides SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, ~~according to claim 4~~, and one or other of the oligonucleotides SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 6, ~~according to claim 5~~, and are advantageously made up of the pair of oligonucleotides SEQ ID No. 3 and SEQ ID No. 6.

15 7. Pairs of oligonucleotide primers, characterized in that the oligonucleotides which make these up are chosen from those:

20 - having a sequence identical to the extent of at least 80%, preferably 90%, and advantageously 95%, to an oligonucleotide made up of a sequence of about 15 to 25 nucleotides, in particular 20 to 25 nucleotides, comprising at least 10 contiguous nucleotides of the following SEQ ID No. 9:

GAGCCTTATCAGTATTAAATTATC

25 - or of the following sequence ID No. 10:

CATTAATGTTATGTACATTA

- or of the following sequence SEQ ID No. 11:

TTTCACGCGGCATGGTAATT

30 - or of the following sequence SEQ ID No. 12:

ATCCAATGAATTTCACCAGG

- or of the following sequence SEQ ID No. 13:

GTCAATGGTCACAGGACATA

- or of the following sequence SEQ ID No. 14:

ATTGACTTGTGGAGTGC

and in particular the following pairs of primers:

SEQ ID No. 9 with SEQ ID No. 10, SEQ ID No. 6 with SEQ ID No. 11, SEQ ID No. 12 with SEQ ID No. 3, SEQ ID No. 13 with SEQ ID No. 14.

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8. Oligonucleotide comprising a minimum of about 15 nucleotides, preferably a minimum of about 20 nucleotides, in which at least a part of its sequence is identical to the extent of at least 80%, preferably 90%, to the following sequence SEQ ID No. 7:

CTTGATAGTATATCTATTATAATTCC

or to the following sequence ID No. 19:

TAARCCGTGGGGGTCGCTATCCAAT

9. Probes, characterized in that they comprise oligonucleotides according to claim 8 or complementary or inverse/complementary oligonucleotides to the oligonucleotides according to claim 8.

10. Process according to ~~one of claims 1 to 3~~ characterized in that the amplification is carried out by the polymerase chain amplification method (PCR), comprising a repetition of the cycle of the following stages:

- Heating of the DNA extracted from the sample of organic matter such that the DNA is separated into two monocatenated strands.

- Hybridization of oligonucleotide primers ~~according to claims 4 to 7~~ with the monocatenated DNA strands at an appropriate temperature, and

30 Elongation of the oligonucleotide primers by a polymerase at an appropriate temperature.

of SEQ ID No.: 1

claim 1

A 11. Process according to ~~one of claims 1 to 3 and 10~~, characterized in that the amplified sequence obtained is detected by hybridization with a probe or by sequencing.

A/5 12. Process according to claim 11, characterized in that the said probe comprises at least in part an oligonucleotide ~~according to one of claims 8 or 9~~ and a marker.

A 13. DNA fragment which can be obtained by the process according to ~~one of claims 1 to 3 and 10~~, characterized in that it comprises about 500 about 100 base pairs.

A 14. Fragment according to claim 13, characterized in that it has a sequence identical to the extent of at least 80%, preferably 90%, and advantageously 95%, to an oligonucleotide made up of a sequence of about 15 to 25 nucleotides contained in the following SEQ ID No. 8:

GCATATAAGCAAGYACATRAYYCTAYAVYAGTACATAAYRCATAYAAT
 TATTRAYYGTACATAGTACATTATRTCAAAYYCATTYCTYRAYARYATATYTAY
 YATATAYYYCYTNCCAYTAGATCACGAGCTTAAYTACCATGCCCGTGAACACC
 ARCAACCCGCTRRGCAGNGGATCCCTCTTCGCTCCGGGCCATARAYYGTG
 GGGGTCGCTATYYARTGAAYTTTAYCAGGCATCTGGTTCTTCAGGGCCA
 TCTCATCTAAARYGTCCATTCTCCTCTTAAATAAGACATCTCGATGGACTAA
 TGRCTAATCAGCCCATGCTCACACACATAACTGTGTYGTACATACATTGGTATTT
 TTTATTTGGGGATGCTTGGACTCAGCTATGGCCGTCAAAGGCCCTGACCCG
 GAGCATCTATTGTAGCTGGACTTAACTGCATCTTGAGCACCAGCATAATGATA
 RGCRTG

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and in particular a fragment of 480 base pairs comprising or made up of SEQ ID No. 8 defined above.

30 15. Fragments according to claim 13, characterized in that they have a sequence identical to the extent of at least 80%, preferably 90%, and advantageously 95%, to an oligonucleotide made up of a sequence of about 15 to 25 nucleotides contained in the

following SEQ ID No. 15:

5
 GAGCCTTATCAGTATTAAATTATCAAAAATCCAATAACTCAACACAGA
 ATTTGCACCCCTAACCAAATATTACAAACACCACTAGCTAACATAACACGCCA
 TACACAGACCACAGAATGAATTACCTACGCAAGGGTAATGTACATAACATTA
 ATG

or in the following SEQ ID NO. 16:

10
 GCATATAAGCAAGTACATGACCTCTATAGCAGTACATAATACATATAATT
 ATTGACTGTACATAGTACATTATGTCAAATTCTTGTAGTATATCTATTA
 TATATTCTTACCAATTAGATCACGAGCTTAATTACCATGCCGCGTGAAA

or in the following SEQ ID No. 17:

15
 ATCCAATGAATTTACCAAGGCATCTGGTTCTTCAGGGCCATCTCAT
 CTAAAACGGTCCATTCTTCTCTRAAAATAAGACATCTCGATGGACTAATGGC
 TAATCAGCCCAGTCTCACACATAACTGTGCTGTACATTTGGTATTTTTA
 TTTTGGGGATGCTTGGACTCAGCTATGCCGTCAAAGGCCCTGACCCGGAGC
 ATCTATTGTAGCTGGACTTAACTGCATCTTGAGCACCAGCATAATGATAAGCA
 TG

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 or in the following sequence SEQ ID No. 18:

25
 GTCAATGGTCACAGGACATAAATTATATTATATCCCCCTTCATAAAA
 ATTCCCCCTTAAATATCTACCAACCACTTTAACAGACTTTCCCTAGATACTT
 ATTAAATTTTACGCTTCAATACTCAATTAGCACTCCAAACAAAGTCAAT

and in particular a fragment of 159 base pairs comprising or made up of SEQ ID No. 15 defined above,

and in particular a fragment of 153 base pairs comprising or made up of SEQ ID No. 16 defined above,

30 and in particular a fragment of 265 base pairs comprising or made up of SEQ ID No. 17 defined above,

and in particular a fragment of 158 base pairs comprising or made up of SEQ ID No. 18 defined above.

claim 1

16. Use of the process according to one of claims 1 to 2 and 10 to 12 for detecting biological matter of bovine origin in products such as: meals used for feeding cattle, composts, manures and dungs, raw, smoked or cooked meats and mixtures of meats, pellets, blood and products based on blood, milk and products based on milk, bone and products based on bone, hides, skins, ivories, furs, horns and products based on horn, guano, faeces, semi-liquid manures, liquid manures, gelatine and products based on gelatine, cosmetic products and products used in the agricultural food industry.

add
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